Optimal foraging by herbivores maintains polymorphism in defence in a natural plant population

AUTHOR(S):

Sato, Yasuhiro; Ito, Koichi; Kudoh, Hiroshi

Optimal foraging by herbivores maintains polymorphism in defence in a natural plant population

Yasuhiro SATO[a,b†*], Koichi ITO[a,c†], and Hiroshi KUDOH[a]

[a] Center for Ecological Research, Kyoto University, Hirano 2-509-3, Otsu, Shiga 520-2113 Japan

[b] Department of Plant Life Sciences, Faculty of Agriculture, Ryukoku University, Yokotani 1-5, Seta Oe-cho, Otsu, Shiga 520-2194, Japan

[c] Department of Psychology, College of Life and Environmental Sciences, University of Exeter, Washington Singer Laboratories, Perry Road, Exeter, EX4 4QG, UK

[†]*equal contribution*

*Author correspondence: Y. Sato

Department of Plant Life Sciences, Faculty of Agriculture, Ryukoku University

Yokotani 1-5, Seta Oe-cho, Otsu, Shiga 520-2194, Japan

Tel: +81-77-599-5660; Fax: +81-77-599-5656

E-mail: sato.yasuhiro.36c@kyoto-u.jp

Running title: Optimal forager maintains plant defence polymorphism

Abstract: 249 words; main text: 5966 words (excluding References); figures: 3; tables: 1.

Supporting information: appendices: 6.

SUMMARY

1. Many species of plants and animals exhibit polymorphism for defensive traits. Adaptive foraging by natural enemies has long been hypothesized to maintain such polymorphism, but this has not been clearly demonstrated in a natural prey or host population.

2. The purpose of this study was to address whether the brassica leaf beetle *Phaedon brassicae* promotes the maintenance of defence polymorphism in the trichome-producing (hairy) and trichomeless (glabrous) morphs of *Arabidopsis halleri* subsp. *gemmifera*. Here, we modelled foraging behaviours of herbivores and demography of the host plant. Then, we estimated the model parameters based on the likelihood of observed data from a natural *A. halleri* population.

3. The patterns of leaf damage to hairy and glabrous plants were well explained when we presumed the optimal diet choice by *P. brassicae*. The observed dynamics in the plant number and morph frequency were well supported by the model with the estimated parameter values. Our numerical analysis showed that the optimal diet choice by *P. brassicae* caused a negative frequency-dependent selection on trichrome dimorphism. The coexistence of two morphs was allowed over a wide range of herbivory pressure and the cost of defence.

4. These results indicate that the optimal diet choice by *P. brassicae* contributes to the coexistence of hairy and glabrous *A. halleri*. While species interaction and stochastic dispersal are both involved in polymorphism dynamics in the field, our findings suggest that the role of consumer behaviours in the maintenance of defence polymorphism may be more important than currently appreciated.

Keywords: *Arabidopsis halleri* subsp. *gemmifera*; Associational effects; Genetic variation; Herbivory; Optimal diet choice; Plant defence

INTRODUCTION

Many plant and animal species develop defence traits against their natural enemies. Genetic variation within species in defence traits has often been reported for both chemical and morphological traits (e.g. Blouw & Hagen 1984; Hauser, Harr & Schlötterer 2001; Hare & Elle 2004; Ruxton, Sherratt & Speed 2004; Wise, Yi & Abrahamson 2009; Moore *et al*. 2014). Polymorphism in defence levels within a prey or host species has been interpreted as a type of Batesian mimicry, the so-called "automimicry" (e.g. Brower 1960; Augner & Bernays 1998; Ruxton, Sherratt & Speed 2004; Svennungsen & Holen 2007; Moore *et al*. 2014). One potential mechanism maintaining dimorphism in defence levels is the negative frequency-dependent selection (also known as apostatic selection) (Sherratt & Harvey 1993; Ruxton, Sherratt & Speed 2004). If this type of selection occurs, the rarer morphs have higher fitness compared to the abundant ones and consequently increase in frequency over time, allowing multiple morphs to coexist within a population. Thus, evolutionary ecologists have often asked how negative frequency-dependent selection may be a consequence of consumer response to polymorphism in defence levels of resources (e.g. Matthews 1977; Ruxton, Sherratt & Speed 2004; Svennungsen & Holen 2007).

Optimal foraging behaviour of consumers has been proposed as a mechanism that can generate negative frequency-dependent selection (e.g. Murdoch 1969; Matthews 1977; Abrams 1982; Holt 1983). For example, the optimal diet choice (ODC) theory predicts that the density of preferred resource alters the optimal level of preference to suboptimal ones so that consumers maximize their energy gain (MacArthur & Pianka 1966; Charnov 1976). Because such a change in preference increases the consumption rate for suboptimal resource as the density of preferred resource reduces, the consumption following ODC potentially drives the negative frequency-dependent selection on resource polymorphism (Abrams 1982;

Holt 1983). Indeed, several experimental studies have shown that such frequency-dependent consumption results in negative frequency-dependent selection between multiple morphs in animal prey (e.g. Brower 1960; Skelhorn & Rowe 2005; Fitzpatrick, Shook & Izally 2009) and consequently maintains the polymorphism in an experimental prey population (Bond & Kamil 1998). However, there is still limited evidence of the contribution of ODC to the maintenance of defence polymorphism in a natural population.

In plant–herbivore interactions, the risk of herbivory in individual plants depends not only on plants' own phenotypes but also on those of the neighbouring plants (e.g. Barbosa *et al.* 2009; Wise, Yi & Abrahamson 2009; Sato & Kudoh 2016; Verschut *et al*. 2016). This phenomenon has been called "associational effects" (Barbosa *et al.* 2009; Underwood, Inouye & Hambäck 2014) and may influence plant's coexistence through frequency-dependent herbivory (Underwood, Inouye & Hambäck 2014). To date, many empirical studies have suggested that herbivore behaviour mediates such associational effects between differentially defended plants (e.g. Hjältén, Danell, & Lundberg 1993; Bergvall & Leimar 2005; Rautio *et al*. 2012; Sato & Kudoh 2016; Verschut *et al*. 2016). For example, if herbivores choose among individual plants growing in a close proximity, this food preference could lead to an increased benefit of the defence, namely associational resistance, for unpalatable plants within patches (Bergvall & Leimar 2005; Sato & Kudoh 2016; Verschut *et al*. 2016; Hahn & Orrock 2016). If herbivores avoid unpalatable plants and prefer to palatable plants in a neighbourhood, associational susceptibility for palatable plants may occur at the same time within a patch (Verschut *et al*. 2016; Hahn & Orrock 2016). Theoretical studies also suggest that such a food choice by herbivores may lead to the associational effects and the coexistence of defended and undefended plants (Holt & Kotler 1987; Ishii & Crawley 2011; Hambäck *et al*. 2014). Little is known, however, about the role of foraging behaviour of

herbivores in associational effects and the maintenance of defence polymorphism.

Several plant species of the genus *Arabidopsis* exhibit dimorphism in trichome production within populations, having both trichome-producing (hereafter, hairy) and trichomeless (hereafter, glabrous) individuals (Hauser, Harr & Schlötterer 2001; Kawagoe *et al*. 2011). *Arabidopsis* trichomes confer resistance to leaf chewing herbivores (Mauricio 1998; Sletvold *et al.* 2010; Sato *et al.* 2014; Sato & Kudoh 2016) but impose a fitness cost on plants (Mauricio 1998; Sletvold *et al.* 2010; Kawagoe *et al.* 2011). In *A. halleri* (L.) O'Kane & Al-Shehbaz subsp. *gemmifera* (Matsum.) O'Kane & Al-Shehbaz [Brassicaceae], the brassica leaf beetle, *Phaedon brassicae* Baly [Coleoptera: Chrysomelidae], preferred glabrous plants when hairy ones were rare and thereby mediated a frequency-dependent damage between hairy and glabrous plants in the field (Sato *et al.* 2014; Sato & Kudoh 2017a). Our laboratory experiment also found that hairy plants incurred a growth cost of defence and the growth rate of each morph was negatively correlated with its own frequency under the defence–growth trade-off (Sato & Kudoh 2016). These findings suggest that the leaf beetle drives associational effects and consequently causes a frequency-dependent selection (Sato & Kudoh 2017a), but this hypothesis has not been tested explicitly.

In this study, we combined theoretical models and empirical data to test the hypothesis that foraging behaviours of *P. brassicae* contribute to the maintenance of trichome dimorphism in natural *A. halleri* populations. Specifically, the following three questions were addressed in this study: (1) Does optimal diet choice (ODC) account for the observed damage by leaf beetles in hairy and glabrous individuals? (2) Can ODC explain the observed patch dynamics of hairy and glabrous plants in the field? (3) Does ODC contribute to the coexistence of hairy and glabrous morphs via negative frequency-dependent selection?

MATERIALS AND METHODS

Study organisms

Arabidopsis halleri is a self-incompatible perennial herb distributed across Japan and the Russian Far East. Hairy individuals of this subspecies produce non-glandular trichomes on their leaf and stem surfaces (Fig. 1a), whereas glabrous plants have no trichomes except on leaf margins (Fig. 1b). The hairy and glabrous phenotypes are associated with allelic variations in a single gene *GL1* (Kawagoe *et al*. 2011) for which glabrousness is considered recessive. Plants have no specialized organs for long-distance seed dispersal and produce clonal rosettes after flowering (Fig. 1d). Our field survey was conducted in a natural *A. halleri* population in central Japan (Taka-cho, Hyogo; 35°06′N, 134°56′E; Sato *et al*. 2014). Thousands of *A. halleri* plants occur in fragmented habitats along a creek, and no Brassicaceae species other than the study species inhabits the field site. *Phaedon brassicae* is abundant in the flowering season and impacts plant fecundity by damaging the flowering stems (Kawagoe & Kudoh 2010).

Phaedon brassicae is a crucifer-feeding agricultural pest distributed across Asia (Ôtake & Funaki 1958; Wang *et al*. 2007). The beetle reaches maturity around 3 weeks after hatching (Wang *et al*. 2007). Adults are flightless and access their host plants by walking (Ôtake & Funaki 1958) (Fig. 1c), and larvae develop on individual plants on which they hatch (Ôtake & Funaki 1958). Thus, adults select their host plants not among but within plant patches, and the damage to individual plants is attributable to host choice by adults during oviposition.

Leaf damage

We first focused on the foraging behaviour of the walking beetle *P. brassicae* in order to explain the pattern of leaf damage in hairy and glabrous plants. Individual *A. halleri* plants are spatially aggregated in the field; hereafter, this pattern of aggregation is denoted "patches". The diameter of each plant patch was less than 1 m at the field site (Sato *et al.* 2014). At this patch scale, adult beetles walk freely among individual plants of *A. halleri* and feed on them. However, since *P. brassicae* are flightless, the neighbouring plant patches are far more distant (mean ± SD = 6.1 ± 3.9 m, n = 48 patches) than the neighbouring plant individuals (mean ± SD = 9.0 ± 5.7 cm, n = 232 plants). Besides, the plant density within a patch does not depend on the patch size as the average distance between neighbouring plants was not significantly correlated with the patch diameter (cm) (r = –0.17, P = 0.25, n = 46 patches). These facts lead us to assume that interpatch movements are much more costly for *P. brassicae* than the intrapatch movements and rarely happen during their host choice; thus, we focused on the optimization of host plant choice by *P. brassicae* at individual level within patches.

We applied the optimal diet choice (ODC) theory (MacArthur & Pianka 1966; Charnov 1976) to model the behavioural response by which *P. brassicae* optimize their foraging depending on the number of hairy and glabrous plants within a patch. The parameters of this ODC model were estimated using the laboratory data on leaf damage in hairy and glabrous plants (Sato & Kudoh 2016) and implemented to predict the patterns of field data on leaf damage (Sato *et al.* 2014).

Theoretical model. We consider a plant population to be composed of spatially isolated patches in which hairy and glabrous plants may co-occur. Herbivores stroll among individual plants within a patch and optimize their foraging behaviour. Because the distance

between neighbouring *A. halleri* plants is independent of the patch size, we assume that the herbivore encounters any plant individual within the patch at a constant rate P. Thus, the encounter rates for glabrous and hairy plants are represented as $PN_g/(N_g+N_h)$ and $PN_h/(N_g+N_h)$, respectively, where N_g denotes the number of glabrous and N_h the number of hairy plants within the patch. We represent the handling time of glabrous and hairy plants for herbivores as H_g and H_h and their energy intake as E_g and E_h, respectively. We assumed that for herbivores glabrous plants are a more profitable resource compared to hairy plants, i.e. $E_g/H_g > E_h/H_h$. According to the ODC model (Charnov 1976), feeding on both glabrous and hairy plants is optimal for herbivores when

$$\frac{E_h}{H_h} > \frac{\frac{PN_g}{N_h+N_g}E_g}{1+\frac{PN_g}{N_h+N_g}H_g}. \qquad \text{Eq. (1)}$$

Otherwise, feeding only on glabrous plants gives herbivores better energy acquisition than feeding on both. Solving Eq. (1) by using the frequency of hairy plants $f = N_h/(N_h+N_g)$, we obtain the threshold frequency of hairy plants at which herbivores should switch their foraging tactics f^*, i.e.

$$f^* = 1-\frac{se}{h-e}, \qquad \text{Eq. (2)}$$

where $e = E_h/E_g$, $h = H_h/H_g$, and $s = 1/PH_g$.

Although the original ODC theory predicts the abrupt change of preferences to a suboptimal resource at $f = f^*$, in nature such a preference will gradually change with the frequency of the suboptimal resource (Stephens & Krebs 1986) because of the incorrect estimation of the frequency of suboptimal plant or the limited knowledge of the environment (McNamara & Houston 1987; Křivan 2010). Thus, we assumed that the probability of

feeding on hairy plants $D(f)$ gradually changes with the frequency of hairy plants f. Following the formula of McNamara and Houston (1987),

$$D(f) = \frac{1}{1+\exp[-\alpha(f - f^*)]}, \qquad \text{Eq. (3)}$$

where α denotes the accuracy of herbivore response; at very large α the $D(f)$ values are the same as those predicted by the original ODC model (i.e. $D(f) = 0$ when $f < f^*$, $D(f) = 1$ when $f \geq f^*$). Note that the probability of feeding on glabrous plants is always one, because the partial preference for glabrous plants always reduces the total energy acquisition.

When herbivores feed on plants as the result of partial preference to hairy plants $D(f)$, the damage by herbivory to glabrous and hairy plants are

$$\frac{\varepsilon}{s+(1-f)+fhD(f)}\frac{M(N_g,N_h)}{N_g+N_h} \quad \text{and} \qquad \text{Eq. (4a)}$$

$$\frac{\varepsilon D(f)}{s+(1-f)+fhD(f)}\frac{M(N_g,N_h)}{N_g+N_h}, \text{ respectively,} \qquad \text{Eq. (4b)}$$

where ε is the coefficient of damage per attack and $M(N_g, N_h)$ is the number of herbivores within the patch (see Appendix S1 in Supporting Information for the derivation). Although the functional shape of $M(N_g, N_h)$ has often been discussed (cf. Hambäck *et al.* 2014), the damage to *A. halleri* was proportional to the number of plants in a patch in our study site (Appendix S2); therefore, in the following analysis we assumed the linear relationship, i.e. $M(N_g, N_h) = m(N_g + N_h)$ (but see Appendix S2 for non-linear analyses). Then, the damages to a glabrous and hairy plant are

$$\frac{v}{s+(1-f)+fhD(f)} \quad \text{and} \qquad \text{Eq. (5a)}$$

$$\frac{vD(f)}{s+(1-f)+fhD(f)}, \text{ respectively,} \qquad \text{Eq. (5b)}$$

where $v = \varepsilon m$.

Data. Two datasets were used to predict herbivory by *P. brassicae*. The laboratory experimental data provided the extent of leaf damages (evaluated as a proportion of lost leaf area) by adult *P. brassicae* under two ratios of hairy to glabrous individuals (3:1 and 1:3) (Sato & Kudoh 2016). The field observational data provided the extent of leaf damage (evaluated as the proportion of leaf area lost to herbivory; scored from 0 to 1.0 at 0.1 increments) in hairy and glabrous plants in a set of circular monitoring plots (1 m in diameter) (Sato *et al.* 2014). The details of the field surveys and experiments are described in previous publications (Sato *et al*. 2014; Sato & Kudoh 2016). The dataset used in the present study is available at the Dryad Digital Repository (Sato, Ito & Kudoh 2017).

Statistical analysis. We first estimated the model parameters of ODC (e, h, s, and α) from the laboratory experimental data on leaf damage (Sato & Kudoh 2016). The leaf damage to individual plants was fitted by a least-square method. For the parameter estimation, we used the 'optim' function implemented in R version 3.2.0 (R Core Team 2015). The initial parameters were set at $e = h = s = \alpha = 1.0$. We first applied the Nelder-Mead method to avoid local optima, and then used the quasi-Newton method (BFGS method in R) to calibrate the parameters. Biases in these parameters were estimated using 95% percentiles of 10,000 bootstrap samplings with replacement of the leaf damage data in individual plants.

To validate the parameters estimated from the laboratory data, we then compared the patterns of leaf damage in hairy and glabrous plants between the model prediction and field data (Sato *et al.* 2014). Given that the herbivore abundance and spatial scales differed between the field and laboratory, we scaled the model prediction to the level of field damage

as: Field damage = β × Damage predicted by the ODC model (using the 'nls' function in R).

Plant demography

Because of the impact of herbivory on plant fecundity and/or mortality, foraging behaviour of *P. brassicae* may affect the demographic dynamics of plants via negative frequency-dependent selection between hairy and glabrous plants. The demography of herbivores in each patch may be translated into herbivory pressure, which in turn influences plant demography. For example, a better energy acquisition will enhance population growth of herbivores, resulting in higher herbivory pressure in the next year. However, our field data showed that the past leaf damage had a negligible influence on the current leaf damage (Appendix S2), and therefore, we focused on the demography of hairy and glabrous *A. halleri* and ignored the population dynamics of *P. brassicae*.

We developed a plant demography model where the plant mortality and/or fecundity were affected by the damage derived from the ODC model, Eq. (5a) and (5b). The parameters of the plant demography model were estimated by fitting the model to the field data on the annual change in the number of hairy and glabrous plants in a set of patches. We then used the estimated parameters to test how ODC by *P. brassicae* contributes to the coexistence of hairy and glabrous plants in natural *A. halleri* populations.

Theoretical model. We considered that the field demography processes of plants were characterized by two sequential events: death and recruitment. In the death event, some of the plants die and thereby the number of plants in a patch decreases. We assumed that the mortality of glabrous and hairy plants increased linearly with the damage caused by herbivory, i.e.

$$m_c + m_d \frac{v}{s + (1 - f) + fhD(f)} \quad \text{and} \qquad \text{Eq. (6a)}$$

$$m_c + m_d \frac{vD(f)}{s + (1 - f) + fhD(f)}, \text{ respectively,} \qquad \text{Eq. (6b)}$$

where m_c is the constant intrinsic mortality, and m_d is the coefficient of additional mortality due to the damage by herbivory. The total number of dead plants is represented as a binominal distribution with the probability given by Eq. (6a) and (6b) (see Appendix S3 for details).

In the recruitment event, new plants are recruited in each patch and thereby the number of plants in the patch increases. We assumed that the fecundity (i.e. the number of seeds or clones) linearly decreased with damage (Eq. (5a) and (5b)) and the cost of trichome production c. Then, the total fecundity of glabrous and hairy plants in a focal patch are

$$R\left[1 - \frac{v}{s + (1 - f) + fhD(f)}\right] n_g \quad \text{and} \qquad \text{Eq. (7a)}$$

$$R\left[1 - \frac{vD(f)}{s + (1 - f) + fhD(f)} - c\right] n_h, \text{ respectively,} \qquad \text{Eq. (7b)}$$

where R is the potential number of offspring per capita, and n_g and n_h are the number of glabrous and hairy plants within the patch, respectively. In *A. halleri*, the plants produce clonal rosettes and seeds are dispersed by gravity. Thus, we assumed that a fraction of produced offspring is dropped within the same patch, while the others reach different patches that are randomly selected from the population. When we represent the former and the latter fraction as 1–d and d, respectively, the number of offspring of glabrous and hairy plants reaching a focal patch are

$$d\tilde{R}_g + (1 - d)R\left[1 - \frac{v}{s + (1 - f) + fhD(f)}\right] n_g \text{ and} \qquad \text{Eq. (8a)}$$

278 $$d\tilde{R}_h + (1-d)R\left[1 - \frac{vD(f)}{s + (1-f) + fhD(f)} - c\right]n_h$$, respectively, Eq. (8b)

279 where $\tilde{R}_g$ is the mean value of Eq. (7a) and $\tilde{R}_h$ is the mean value of Eq. (7b) in the population.
280 We assumed that all the offspring that reached the focal patch will establish and mature in
281 that patch (However, our results were unchanged even when we incorporated the effect of
282 patch size on the invasion difficulty; see Appendix S4.). Although the distribution of the
283 number of newly established plants is uncertain, we approximate the probability distribution
284 of the number of established individuals by a Poisson distribution with the mean given by Eq.
285 (8a) and (8b).

286 In *A. halleri*, individual plants reproduce once a year in spring (Kawagoe & Kudoh
287 2010), while the death event occurs throughout the year. Our field observations were
288 conducted at the end of reproductive season of each year (mid-May; see the next subsection).
289 Thus, the annual life cycle of *A. halleri* can be represented by a single recruitment event
290 followed by a single death event. Consequently, we can quantify the occurrence probability
291 of annual demographic changes by summing up the probability of all possible combinations
292 for the number of dead and recruited plants, which follow a binominal distribution (with the
293 probability as defined by Eq. (6a) and (6b)) and a Poisson distribution (with the mean defined
294 by Eq. (8a) and (8b)), respectively (see Appendix S3 for details of the derivation).
295
296 ***Data.*** Once a year from 2013 to 2016, we randomly selected 80 plant patches and set
297 circular monitoring plots to count the number of all hairy and glabrous plants in the plots.
298 Given that the number of plants within the circular area approached a plateau at 1-m diameter
299 in this site (Sato *et al*. 2014), the diameter of circular plots was set at 1 m to monitor patch
300 size dynamics. The survey was conducted in the late flowering season (mid-May). Leaf

damages during this study period fully reflected the level of herbivory on individual plants as it occurred immediately after the spring outbreak of *P. brassicae*. We surveyed ca. 80 plots per year and obtained 243 measurements of their yearly dynamics. Given the clonality of *A. halleri*, a plant with no vegetative connection with others was designated as an individual in this survey.

Additionally, we used laboratory data on the biomass (mg) of hairy and glabrous plants to compare the estimated cost of defence c with our previous evidence on the growth cost of trichomes (Sato & Kudoh 2016). Potted plants were cultivated for 6 months in an indoor space without herbivory. The intrinsic growth cost for hairy plants was calculated as [ln(median glabrous biomass) – ln(median hairy biomass)] / ln(median glabrous biomass). The details regarding the cultivation are described in Sato and Kudoh (2016). The dataset used in the present study is available at the Dryad Digital Repository (Sato, Ito & Kudoh 2017).

Statistical analysis. Using the estimated parameters of the ODC model (e, h, s, and α), we then estimated the six parameters (v, R, c, d, m_c, and m_d) of the plant demography model. The field patch data on the number of hairy and glabrous plants observed from 2013 to 2015 were used to quantify the likelihood of the observed field dataset pertaining to annual patch dynamics in our plant demography model (see Appendix S3 for derivation). To increase the log-likelihood of observed data, we ran eight independent chains of Markov chain Monte Carlo (MCMC) with 10^6 iterations, 10^3 thinning interval, and 10^5 burn-in period, starting from the following initial parameters: $v = 0.0$, $R = 1.0$, $c = 0.0$, $d = 0.5$, $m_c = 0.5$, and $m_d = 0.01$. The mortality due to herbivory m_d was estimated at an exponential scale to examine a range of positive values. Proposal parameters were generated following a normal distribution

N(μ_t, σ^2), where μ_t denotes the parameter value at the current iteration, and σ is set at 0.25 for v and R and at 0.025 for c, d, m_c, and m_d. These proposals were accepted/rejected following the Metropolis algorithm (Hastings 1970). The convergence of MCMCs was assessed by $\hat{R}$ statistic at < 1.1 (Plummer *et al*. 2006). If parameters were estimated to nearly zero, the $\hat{R}$ statistic was calculated after excluding such ineffective parameters. The present modelling and parameter estimation were conducted using the Rcpp (Dirk & Francois 2011), BH (Dirk, Emerson & Kane 2015), coda (Plummer *et al*. 2006) package, and Rtools version 3.3.0.1958 (available from: https: //cran.r-project.org/bin/windows/Rtools/). The R code is available at the Dryad Digital Repository (Sato, Ito & Kudoh 2017).

RESULTS

Prediction of leaf damage patterns

We first estimated the parameters of our ODC model by fitting the predicted damage, Eq. (5a) and (5b), to the laboratory data. Glabrous plants were a more profitable resource for *P. brassicae* than hairy plants as shown by the greater handling time (h = 1.82; Table 1a) and lower energy intake (e = 0.58; Table 1a) of hairy plants. Given the set of the estimated handling time, energy intake, searching time, and response accuracy in the laboratory (Table 1a), our ODC model predicted that *P. brassicae* avoided hairy plants when hairy plants were rare, but fed on both hairy and glabrous plants when hairy ones were abundant (solid lines and circles in Fig. 2a, b). In other words, the defensive role of producing hairs is effective when the frequency of glabrous plants is increased, whereas glabrous plants receive weak effects from neighbours. This result shows that the ODC by *P. brassicae* causes the associational effects.

Our ODC model with the estimated parameters (Table 1a) provided a comparable pattern with the observed trends in the field, as the intervals of prediction overlapped between the model (solid lines in Fig. 2a, b) and field data (dashed lines in Fig. 2a, b) on leaf damages in the two morphs. Hairy plants were less damaged when they were rare, whereas the damage to glabrous plants did not depend on the frequency of the two morphs in the field (dashed lines and pale plots in Fig. 2a, b). These results indicate that the ODC by *P. brassicae* accounts for the pattern of leaf damage to hairy and glabrous plants.

Prediction of plant demography

Based on the estimated parameters of the ODC model (Table 1a), we next examined the sets of six parameters of the plant demography model that increase the likelihood of observed field data (Table 1b). The herbivory pressure v, cost of defence c, interpatch dispersal d, and intrinsic mortality m_c were involved in the field demography of hairy and glabrous plants, whereas the mortality due to herbivory m_d was negligible at nearly zero (Table 1b). The growth cost for hairy plants directly compiled from the laboratory biomass data showed a smaller but comparable level of the defence cost (empirical c = 0.09 [0.02–0.13], bootstrap median with 95% confidence interval [CI]) with the estimated cost c (c = 0.22 [0.15–0.24], median with 95% CI; Table 1b). Approximately 10% of the recruitment process was attributable to the interpatch dispersal (d = 0.10 [0.08–0.13]; Table 1b). Convergence of MCMCs was confirmed by $\hat{R}$ statistic ($\hat{R}$ = 1.015).

Then, we tested the validation of our estimation by forecasting the test data. Using the parameters estimated from the data obtained in the first three years (2013 to 2015), we numerically simulated plant demography for 2015 to 2016 and then compared the predicted dynamics with the morph-frequency and patch size observed in 2016. The probability density

of the frequency of hairy plants in a patch was comparable with that observed in 2016 (Fig. 2c). The number of *A. halleri* plants observed in 2016 ranged within those predicted by the model (Fig. 2d). Although we assumed ODC by *P. brassicae*, the observed demography might be predicted without ODC. Therefore, we estimated the model parameters assuming the absence of ODC, i.e. no difference between glabrous and hairy plants for herbivores (h = e = 1). This model selection showed that the model without ODC was less fitted to the observed demography than that with ODC (difference of the deviance information criterion, ΔDIC = 9.95; see also Appendix S5). These results indicate that the morph-frequency and patch size dynamics are predictable by the plant demography model incorporating ODC.

We further assessed whether the estimated model showed a similar morph-frequency as observed in the natural population. The frequency of hairy plants at the equilibrium state was derived by analysing the condition under which fitness of the two morphs becomes equal (by solving Eq. (7a) and (7b); see Appendix S6 for derivation). We analysed the equilibrium frequency and its confidence intervals using the parameter sets sampled from MCMCs. These parameter sets predicted the intermediate frequency of hairy plants (predicted f = 0.53 [0.45–0.62], median [95% CI]), and this interval of predicted frequency was overlapped with the observed frequency of hairy plants in the natural population (observed f = 0.52 [0.47–0.58] estimated by 10,000 bootstrap replicates on plant patches surveyed in 2016). Thus, the estimated model provides quantitative prediction of the observed frequency in natural population.

Coexistence of hairy and glabrous plants

To test whether the model with estimated parameters (Table 1) predicts a negative frequency-dependent selection, we asked (1) whether rarer morphs have higher fitness than

397 abundant ones and (2) whether frequencies of rarer morphs increase over time. First, we
398 calculated the relative fitness of hairy and glabrous plants with respect to the frequency of
399 hairy plants in the population (Fig. 3a). The model predicts that relative fitness of one morph
400 increases as it becomes relatively rare (Fig. 3a), showing negative frequency-dependent
401 selection between the two morphs. Second, because the result based on the relative fitness
402 does not include the effect of patch structure at the population scale, we performed numerical
403 simulations to confirm negative frequency-dependent selection at such a population scale. We
404 constructed a virtual plant population whose patch size followed the Poisson distribution with
405 a mean patch size observed in the natural population ($N_g + N_h = 10.03$) and simulated the
406 patch size dynamics by applying the model with the estimated parameters (Table 1). The
407 simulated dynamics showed that the frequency of hairy plants at the population scale
408 approached the equilibrium frequency over time (blue points in Fig. 3b) even when the
409 meta-patch structure was taken into consideration. Contrarily, a numerical simulation without
410 any effects of ODC (i.e. $h = e = 1$) did not show the frequency-dependent selection between
411 the two morphs (red points in Fig. 3b). These results indicate that ODC by leaf beetles
412 promotes the maintenance of trichome dimorphism via negative frequency-dependent
413 selection.

414 Finally, we analysed the conditions under which the herbivory pressure v and
415 defence cost c allow hairy and glabrous plants to coexist within a population (Fig. 3c; see
416 also Appendix S6 for derivation). The estimated range of herbivory pressure v and the
417 defence cost c was included within the predicted region where hairy and glabrous plants
418 coexist (Fig. 3c). This region for coexistence was broader than that for extinction of one
419 morph. This analysis indicates that, in the presence of ODC by leaf beetles, hairy and
420 glabrous plants likely coexist even if the herbivory pressure and cost of defence vary across a

421 wide range.

422

423 **DISCUSSION**

424

425 **Optimal forager promotes the maintenance of polymorphism**

426 How natural enemies govern prey diversity is an intriguing and long-standing question in
427 trophic interactions (e.g. Brower 1960; Murdoch 1969; Ruxton, Sherratt & Speed 2004).
428 Although several studies to date have shown the importance of natural enemies for
429 coexistence of multiple prey (or host) species (Ishii & Shimada 2012) or morphs (Bond &
430 Kamil 1998) in the laboratory, quantitative evidence in natural populations remains limited.
431 Here, we have shown that optimal diet choice (ODC) by herbivores is a crucial mechanism
432 stabilizing the coexistence of hairy and glabrous plants in a natural population based on the
433 following three results. First, the pattern of leaf damage to hairy and glabrous plants was
434 explained by ODC by the leaf beetles. Second, the model incorporating ODC better predicted
435 plant demography than the model without ODC. Third, the numerical analysis and simulation
436 revealed that ODC by the leaf beetle has driven a negative frequency-dependent selection that
437 maintains the dimorphism of hairy and glabrous plants. These results present one of few field
438 examples that demonstrate the stabilizing role of natural enemies in defence polymorphism.
439 The prediction of ODC wherein consumers feed on suboptimal resource when
440 preferred resource is rare has been supported for many species (Pyke, Pulliam & Charnov
441 1977; Sih & Christensen 2001), especially those feeding on sessile prey (Sih & Christensen
442 2001). This prediction is applicable to adult host choice and consequent larval performance in
443 herbivorous insects (Jaenike 1978; Scheirs & Bruyn 2002). Hairy plants are considered
444 suboptimal for *P. brassicae* since larvae grow better when they are fed glabrous leaves than

445 hairy leaves (Sato *et al*. 2014). Given that larvae of *P. brassicae* have no opportunity to
446 actively choose host plants (Ôtake & Funaki 1958), plant damages are attributable to adult
447 host choice followed by larval feeding. Although the proximate mechanisms by which adult
448 beetles recognise hairy and glabrous plants have yet to be elucidated, ODC is a plausible
449 mechanism underlying the pattern of plant damage.

450 Qualitatively speaking, the maintenance of defence polymorphism under
451 herbivore-mediated interactions has often been discussed in the context of game theory that
452 assumes the intrinsic cost and associational benefits of the defence among defended and
453 undefended plants (Augner & Bernays 1998; Rautio *et al*. 2012). There is a growing body of
454 evidence on the cost of physical and chemical defence against herbivores (e.g. Mauricio
455 1998; Hare & Elle 2004; Sletvold *et al*. 2010). More importantly, recent studies on
456 associational effects have shown that food preference by herbivores causes associational
457 resistance for unpalatable plants and simultaneously leads to associational susceptibility for
458 plants within a patch (Verschut *et al*. 2016; Hahn & Orrock 2016). In contrast, ODC by the
459 leaf beetle *P. brassicae* predicts intrapatch associational resistance for hairy plants, but not
460 associational susceptibility for glabrous plants. This is because the handling time on hairy
461 plants is much longer than that on glabrous plants (h = 1.82; Table 1a) and thereby the
462 existence of hairy plants decreases the attack rate to glabrous plants. As discussed in the
463 former paragraph, optimal foragers are expected to feed on unpalatable food only when
464 palatable food becomes rare (MacArthur & Pianka 1966; Charnov 1976). In terms of ODC,
465 our present study explains why only the associational resistance for unpalatable plants occur
466 within a patch.

467 Remarkably, the coexistence of two morphs was widely predicted across the
468 parameter region of the defence cost c or herbivory pressure h. For example, under the

estimated parameters, the coexistence can still be possible even if the defence reduces the reproductive success by almost 50% (Fig. 3c). Moreover, according to the estimated parameters (Table 1a), the preference for hairy plants changes very gradually with the frequency of the hairy plants, i.e. the probability of feeding on hairy plants $D(f)$ is expected to be 0.43 and 0.90 when the patch is filled by glabrous plant ($f = 0$) and hairy plant ($f = 1$), respectively. Therefore, neither the subtle balance between cost and benefit of the defence nor the high accuracy of herbivore foraging is a necessary condition for the coexistence of two morphs. This line of analysis implies that the stabilizing role of ODC by herbivores in the maintenance of defence polymorphism may be widespread in plant–herbivore and other trophic interactions.

Disentangling species interaction under complex field environment

Spatial heterogeneity within a single population is hypothesized to be important as plants are incapable of escaping from herbivores (Ishii & Crawley 2011; Hambäck *et al*. 2014). Considering a meta-patch structure and the intrapatch effects of herbivore foraging, we were able to evaluate the relative importance of interpatch dispersal and intrapatch herbivory in the morph-frequency dynamics. The result that showed that the intrapatch dispersal of plants was predominant compared to the interpatch dispersal (as indicated by the estimated proportion of interpatch dispersal, $d = 0.10$) seems convincing, because *A. halleri* plants disperse their offspring near a maternal plant by clonal propagation or seed dispersal by gravity. Separating the plant life cycle into the two stages, we also uncovered the impact of herbivory on the recruitment but not on the death stage. This result agrees with the fact that *P. brassicae* become abundant in the recruitment season, decreasing plant fecundity (Kawagoe & Kudoh 2010). Given that *A. halleri* individuals are rarely killed by infestation by *P. brassicae* alone

493 (only one dead out of 120 plants as reported by Sato & Kudoh 2016), it was conceivable that
494 herbivory did not affect mortality.

495 Although our study found that foraging behaviour of *P. brassicae* contributes to the
496 maintenance of polymorphism in *A. halleri*, this does not necessarily indicate the absence of
497 other factors affecting the frequency of defended plants within a population. Co-occurrence
498 of defended and undefended morphs might also be allowed by spatiotemporal variation in
499 herbivory pressure and migration among populations (Hare & Elle 2004; Andrew *et al*. 2007;
500 Sato & Kudoh 2017b). The frequencies of hairy and glabrous plants and the prevalent
501 herbivore species indeed vary among *A. halleri* populations (Sato & Kudoh 2015; Sato &
502 Kudoh 2017b). Notably, the present modelling approach enables us to quantify the stability of
503 the morph frequency caused by ODC (Fig. 3b) or to perform a model selection that compares
504 the predictability among multiple models (e.g. DICs). Although temporal dynamics between
505 the current and past herbivory pressure were unclear in our 4-year survey (Appendix S2), this
506 effect might be significant in long-term data. Further understanding of the relative importance
507 of multiple factors responsible for the co-occurrence of defended and undefended morphs
508 will be possible by analysing the extended model combined with the long-term demography
509 data from multiple plant populations.
510
511 **Conclusion**
512 Our present findings suggest that adaptive foraging by natural enemies may play a more
513 important role in stabilizing defence polymorphism in the wild than previously thought.
514 Recent studies have begun to adopt a likelihood approach to detect the ongoing
515 frequency-dependent selection from the time-series polymorphism dynamics (Rouzic *et al*.
516 2015) or to test the mechanisms underlying the coexistence of plant genotypes (Miller &

517 Rudgers 2014). Although our present study focused on *A. halleri* and *P. brassicae*, this type
518 of modelling may be applied to other systems. Several studies on plant–herbivore interaction
519 suggest that scales of associational effects depend on spatial structure of herbivores and
520 plants (Hjältén, Danell, & Lundberg 1993; Sato & Kudoh 2015; Verschut *et al*. 2016). Now
521 that evidence for apparent interaction has been accumulated in various trophic interactions
522 (see Ruxton, Sherratt & Speed 2004 for animals; Barbosa *et al*. 2009 for plants), joint
523 approaches using optimality modelling and empirical data will enable us to address how
524 behavioural basis of natural enemies impacts prey diversity across ecosystems.
525

526 **AUTHOR CONTRIBUTIONS**

527 YS collected and analysed the data. KI developed the mathematical models. YS, KI, and HK
528 conceived the study and wrote the paper.
529

530 **ACKNOWLEDGEMENTS**

531 The authors thank Dr. Andrew D. Higginson for providing helpful comments on an earlier
532 draft of this manuscript. This study was supported by JSPS research fellowship for young
533 scientists to Y. Sato (16J30005) and K. Ito (14J00472), and JSPS Grant-in-Aid for Scientific
534 Research (S) to H. Kudoh (26221106). No conflicts of interests are declared by the authors.
535

536 **DATA ACCESSIBILITY**

537 Source codes and data deposited in the Dryad Digital Repository:
538 http://dx.doi.org/10.5061/dryad.pn088 (Sato, Ito & Kudoh 2017)
539

540 **REFERENCES**

541 Abrams, P. A. (1982) Functional responses of optimal foragers. *American Naturalist*, **120**,
542 382–390.
543 Andrew, R.L., Peakall, R., Wallis, I.R. & Foley, W.J. (2007) Spatial distribution of defense
544 chemicals and markers and the maintenance of chemical variation. *Ecology*, **88**,
545 716–728.
546 Augner, M. & Bernays, E.A. (1998) Plant defence signals and Batesian mimicry.
547 *Evolutionary Ecology*, **12**, 667–679.
548 Barbosa, P., Hines, J., Kaplan, I., Martinson, H., Szczepaniec, A. & Szendrei, Z. (2009)
549 Associational resistance and associational susceptibility: having right or wrong
550 neighbors. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 1–20.
551 Bergvall, U.A. & Leimar, O. (2005) Plant secondary compounds and the frequency of food
552 types affect food choice by mammalian herbivores. *Ecology*, **86**, 2450–2460.
553 Blouw, D. & Hagen, D. (1984) The adaptive significance of dorsal spine variation in the
554 fourspine stickleback, *Apeltes quadracus*. III. correlated traits and experimental
555 evidence on predation. *Heredity*, **53**, 371–382.
556 Bond, A.B. & Kamil, A.C. (1998) Apostatic selection by blue jays produces balanced
557 polymorphism in virtual prey. *Nature*, **395**, 594–596.
558 Brower, J.V.Z. (1960) Experimental studies of mimicry. IV. The reactions of starlings to
559 different proportions of models and mimics. *American Naturalist*, **94**, 271–282.
560 Charnov, E.L. (1976) Optimal foraging: attack strategy of a mantid. *American Naturalist*, **110**,
561 141–151.
562 Dirk, E. & Francois, R. (2011) Rcpp: Seamless R and C++ Integration. *Journal of Statistical*
563 *Software*, **40**, 1–18.
564 Dirk, E., Emerson, J.W. & Kane, M.J. (2015) BH: Boost C++ Header Files. R package
565 version 1.60.0-1. http://CRAN.R-project.org/package=BH
566 Fitzpatrick, B.M., Shook, K. & Izally, R. (2009) Frequency-dependent selection by wild birds
567 promotes polymorphism in model salamanders. *BMC Ecology*, **9**, 1.
568 Hahn, P.G. & Orrock J.L. (2016) Neighbor palatability generates associational effects by
569 altering herbivore foraging behavior *Ecology*, **97**, 2103–2111.
570 Hambäck, P.A., Inouye, B.D., Andersson, P. & Underwood, N. (2014) Effects of plant
571 neighborhoods on plant–herbivore interactions: resource dilution and
572 associational effects. *Ecology*, **95**, 1370–1383.

573 Hare, J.D. & Elle, E. (2004) Survival and seed production of sticky and velvety *Datura*
574 *wrightii* in the field: a five-year study. *Ecology*, **85**, 615–622.
575 Hastings, W.K. (1970) Monte Carlo sampling methods using Markov chains and their
576 applications. *Biometrika*, **57**, 97–109.
577 Hauser, M.T., Harr, B. & Schlötterer, C. (2001) Trichome distribution in *Arabidopsis thaliana*
578 and its close relative *Arabidopsis lyrata*: molecular analysis of the candidate
579 gene *GLABROUS1*. *Molecular Biology and Evolution*, **18**, 1754–1763.
580 Hjältén, J., Danell, K. & Lundberg, P. (1993) Herbivore avoidance by association: vole and
581 hare utilization of woody plants. *Oikos*, **68**, 125–131.
582 Holt, R.D. (1983) Optimal foraging and the form of the predator isocline. *American*
583 *Naturalist*, **122**, 521–541.
584 Holt, R.D. & Kotler, B.P. (1987) Short-term apparent competition. *American Naturalist*, **130**,
585 412–430.
586 Ishii, R. & Crawley, M.J. (2011) Herbivore-induced coexistence of competing plant species.
587 *Journal of Theoretical Biology*, **268**, 50–61.
588 Ishii, Y. & Shimada, M. (2012) Learning predator promotes coexistence of prey species in
589 host-parasitoid systems. *Proceedings of the National Academy of Sciences USA*,
590 **109**, 5116–5120.
591 Jaenike, J. (1978) On optimal oviposition behavior in phytophagous insects. *Theoretical*
592 *Population Biology*, **14**, 350–356.
593 Kawagoe, T. & Kudoh, H. (2010) Escape from floral herbivory by early flowering in
594 *Arabidopsis halleri* subsp. *gemmifera*. *Oecologia*, **164**, 713–720.
595 Kawagoe, T., Shimizu, K.K., Kakutani, T. & Kudoh, H. (2011) Coexistence of trichome
596 variation in a natural plant population: a combined study using ecological and
597 candidate gene approaches. *PLoS ONE*, **6**, e22184.
598 Křivan, V. (2010) Evolutionary stability of optimal foraging: Partial preferences in the diet
599 and patch models. *Journal of Theoretical Biology*, **267**, 486–494.
600 MacArthur, R.H. & Pianka, E.R. (1966) On optimal use of a patchy environment. *American*
601 *Naturalist*, **100**, 603–609.
602 McNamara, J.M. & Houston, A.I. (1987) Partial preferences and foraging. *Animal Behaviour*,
603 **35**, 1084–1099.
604 Matthews, E.G. (1977) Signal-based frequency-dependent defense strategies and the

evolution of mimicry. *American Naturalist*, **111**, 213–222.

Mauricio, R. (1998) Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. *American Naturalist*, **151**, 20–28.

Miller, T.E.X. & Rudgers, J.A. (2014) Niche differentiation in the dynamics of host-symbiont interactions: symbiont prevalence as a coexistence problem. *American Naturalist*, **183**, 506–518.

Moore, B.D., Andrew, R.L., Külheim, C. & Foley, W.J. (2014) Explaining intraspecific diversity in plant secondary metabolites in an ecological context. *New Phytologist*, **201**, 733–750.

Murdoch, W.W. (1969) Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecological Monographs*, **39**, 335–354.

Ôtake, A. & Funaki, S. (1958) The distribution of *Phaedon brassicae* Baly, with special reference to influences of the dispersal of the adults upon the distribution of their next generation. *Bullutein of Shimane Agricultural University*, **6**, 107–116

Plummer, M., Best, N., Cowles, K. & Vines, K. (2006) CODA: Convergence diagnosis and output analysis for MCMC. *R News*, **6**, 7–11.

Pyke, G.H., Pulliam, H.R. & Charnov, E.L. (1977) Optimal foraging: a selective review of theory and tests. *Quarterly Review of Biology*, **52**, 137–154.

R Core Team. (2015) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rautio, P., Bergvall, U.A., Tuomi, J., Kesti, K. & Leimar, O. (2012) Food selection by herbivores and neighbourhood effects in the evolution of plant defences. *Annales Zoologici Fennici*, **49**, 45–57.

Rouzic, A.L., Hansen, T.F., Gosden, T.P. & Svensson, E.I. (2015) Evolutionary time-series analysis reveals the signature of frequency-dependent selection on a female mating polymorphism. *American Naturalist*, **185**, E182–E196.

Ruxton, G.D., Sherratt, T.N. & Speed, M.P. (2004) *Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry*. Oxford University Press, Oxford, UK.

Sato, Y., Kawagoe, T., Sawada, Y., Hirai, M.Y. & Kudoh, H. (2014) Frequency-dependent herbivory by a leaf beetle, *Phaedon brassicae*, on hairy and glabrous plants of *Arabidopsis halleri* subsp. *gemmifera*. *Evolutionary Ecology*, **28**, 545–559.

Sato, Y. & Kudoh, H. (2015) Tests of associational defence provided by hairy plants for

637 glabrous plants of *Arabidopsis halleri* subsp. *gemmifera* against insect herbivores.
638 *Ecological Entomology*, **40**, 269–279.
639 Sato, Y. & Kudoh, H. (2016) Associational effects against a leaf beetle mediate a minority
640 advantage in defense and growth between hairy and glabrous plants.
641 *Evolutionary Ecology*, **30**, 137–154.
642 Sato, Y. & Kudoh, H. (2017a) Herbivore-mediated interaction promotes the maintenance of
643 trichome dimorphism through negative frequency-dependent selection. *American*
644 *Naturalist*, **190**, E67–E77.
645 Sato, Y. & Kudoh, H. (2017b) Fine-scale frequency differentiation along a herbivory gradient
646 in the trichome dimorphism of a wild *Arabidopsis*. *Ecology and Evolution*, **7**,
647 2133–2141.
648 Sato, Y., Ito, K. & Kudoh, H. (2017) Data from: Optimal foraging by herbivores maintains
649 polymorphism in defence in a natural plant population. *Dryad Digital Repository*,
650 doi:10.5061/dryad.pn088
651 Scheirs, J. & De Bruyn, L. (2002) Integrating optimal foraging and optimal oviposition
652 theory in plant-insect research. *Oikos*, **96**, 187–191.
653 Sherratt, T.N. & Harvey, I.F. (1993) Frequency-dependent food selection by arthropods: a
654 review. *Biological Journal of the Linnean Society*, **48**, 167–186.
655 Sih, A. & Christensen, B. (2001) Optimal diet theory: when does it work, and when and why
656 does it fail? *Animal Behaviour*, **61**, 379–390.
657 Skelhorn, J. & Rowe, C. (2005) Frequency-dependent taste-rejection by avian predation may
658 select for defence chemical polymorphisms in aposematic prey. *Biology Letters*,
659 **1**, 500–503.
660 Sletvold, N., Huttunen, P., Handley, R., Kärkkäinen, K. & Ågren, J. (2010) Cost of trichome
661 production and resistance to a specialist insect herbivore in *Arabidopsis lyrata*.
662 *Evolutionary Ecology*, **24**, 1307–1319.
663 Stephens, D.W. & Krebs, J.R. (1987) *Foraging Theory* (1st ed.). Princeton University Press,
664 Princeton, NJ, USA.
665 Svennungsen, T.O. & Holen, Ø.H. (2007) The evolutionary stability of automimicry.
666 *Proceedings of the Royal Society B: Biological Sciences*, **274**, 2055–2063.
667 Underwood, N., Inouye, B.D. & Hambäck, P.A. (2014) A conceptual framework for
668 associational effects: When do neighbors matter and how would we know?

Quarterly Review of Biology, **89**, 1–19.

Verschut, T.A., Becher, P.G., Anderson, P. & Hambäck, P.A. (2016) Disentangling associational effects: both resource density and resource frequency affect search behaviour in complex environments. *Functional Ecology*, **30**, 1826–1833.

Wang, X.-P., Xue, F.-S., Tan, Y.-Q. & Lei, C.-L. (2007) The role of temperature and photoperiod in diapause induction in the brassica leaf beetle, *Phaedon brassicae* (Coleoptera: Chrysomelidae). *European Journal of Entomology*, **104**, 693–697.

Wise, M.J., Yi, C.G. & Abrahamson, W.G. (2009) Associational resistance, gall-fly preferences, and a stem dimorphism in *Solidago altissima*. *Acta Oecologica*, **35**, 471–476.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1 Derivation of the damage

Appendix S2 Total amount of herbivory

Appendix S3 Derivation of the likelihood

Appendix S4 Influence of patch size

Appendix S5 The importance of ODC

Appendix S6 Condition for the coexistence

691 **Table 1** Estimated parameters of the herbivore behaviour and plant demography model.
692 **(a)** Least-square estimates for four parameters responsible for leaf damage to hairy and
693 glabrous plants in the laboratory. The optima estimated by the Nelder-Mead and
694 quasi-Newton method are shown with bootstrap confidence intervals (CIs).

Parameter	Description	Estimate	Bootstrap 95% CI	
			Lower	Upper
h	Handling time	1.81	1.39	2.58
e	Energy intake	0.58	0.48	1.28
s	Searching time	1.88	1.56	2.34
α	Response accuracy	2.52	1.58	3.05

695
696 **(b)** Markov Chain Monte Carlo estimates for six parameters determining plant demography
697 in the field. The median and 95% lower and upper percentiles are shown for parameter
698 distributions.

Parameter	Description	Percentiles		
		Median	2.5%	97.5%
v	Herbivory pressure	2.57	2.07	2.72
R	Intrinsic growth rate	12.87	5.61	21.84
c	Cost of defence	0.22	0.15	0.24
d	Proportion of interpatch dispersal	0.10	0.08	0.13
m_c	Intrinsic mortality	0.75	0.69	0.80
m_d	Mortality due to herbivory	$<10^{-8}$	$<10^{-36}$	0.03
---	*Log-Likelihood*	*-730.3*	*-735.2*	*-728.4*

699
700
701
702
703
704

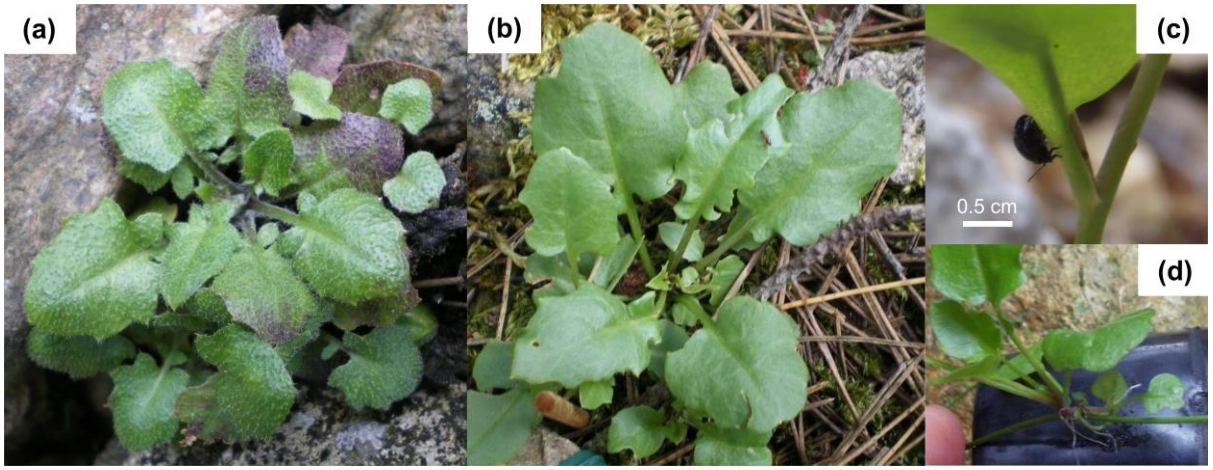


Figure 1 Photographs of *Arabidopsis halleri* subsp. *gemmifera* and *Phaedon brassicae*: (a) hairy plant; (b) glabrous plant; (c) adult beetle; and (d) clonal rosette producing roots.

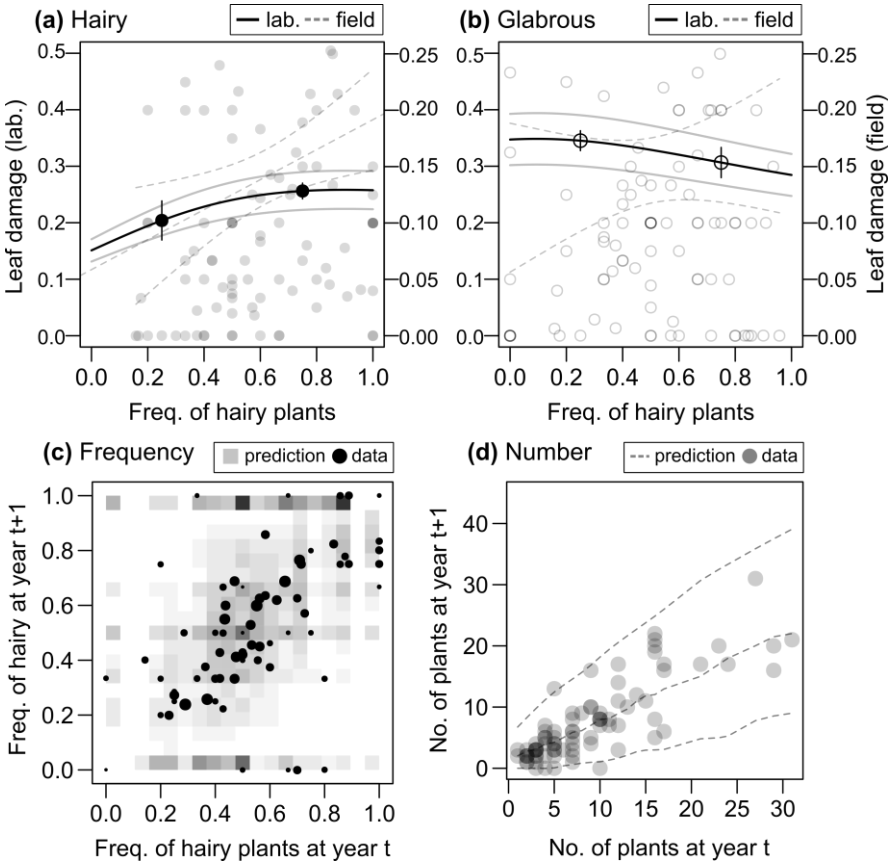


Figure 2 Prediction of leaf damage patterns and demography of hairy and glabrous plants. Leaf damage (evaluated by proportion of the lost leaf area) to hairy (a) or glabrous (b) plants predicted by the optimal diet choice (left axis) and observed in the field (right axis). Solid curves and circles indicate the prediction (±95% CI) and average damage (±SE) in the laboratory (lab.), respectively. Dashed curves and pale circles show linear prediction (±95% CI) and average damage in the field. Prediction of the morph-frequency (c) and patch size (d) dynamics observed from 2015 to 2016. (c) The plot size corresponds to the number of plants in a patch observed in 2015. The darkness of the squares represents the probability density of the simulated frequency changes. (d) Dashed lines indicate the median and 95% prediction intervals of the model simulation.

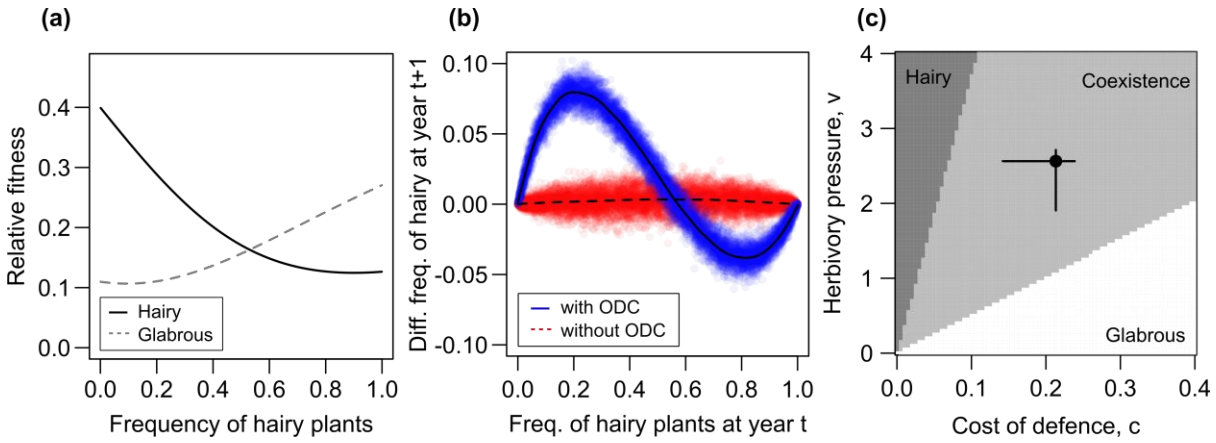


Figure 3 Negative frequency-dependent selection and the coexistence of hairy and glabrous plants as shown by the estimated parameters. (a) Relative fitness between hairy (solid line) and glabrous (dashed line) plants based on Eq. (S9a) and (S9b) in Appendix S6. (b) Population level dynamics of the morph-frequency simulated by models with (blue plots; solid line) or without (red plots; dashed line) the optimal diet choice (ODC). Trend lines are added using a smooth spline method. A single point indicates a simulated plant population consisting of 1,000 patches, and the simulation was repeated 100 times for each frequency. (c) Condition for the coexistence of hairy and glabrous plants under combinations of the herbivory pressure v and defence cost c. Circles and bars indicate the median and 95% percentiles of the estimated v and c.

Appendix S1. Derivation of the damage by herbivory

According to the optimal diet choice (ODC) model (Charnov 1976), the attack rate of a herbivore on glabrous and hairy plants is

$$\frac{P\frac{N_g}{N_g+N_h}}{1+P\frac{N_g}{N_g+N_h}H_g+P\frac{N_h}{N_h+N_h}D(f)H_h} \quad \text{and} \qquad \text{Eq. (S1a)}$$

$$\frac{P\frac{N_h}{N_g+N_h}D(f)}{1+P\frac{N_g}{N_g+N_h}H_g+P\frac{N_h}{N_h+N_h}D(f)H_h}, \text{ respectively.} \qquad \text{Eq. (S1b)}$$

By using s, f, and h, we can rewrite Eq. (S1a) and (S1b) as

$$\frac{1}{H_g}\frac{1-f}{s+(1-f)+fhD(f)} \quad \text{and} \qquad \text{Eq. (S2a)}$$

$$\frac{1}{H_g}\frac{fD(f)}{s+(1-f)+fhD(f)}, \text{ respectively.} \qquad \text{Eq. (S2b)}$$

By dividing Eq. (S2a) and (S2b) by N_g and N_h, respectively, we obtain the frequency of attacks by the focal herbivore on glabrous and hairy plants, i.e.

$$\frac{1}{H_g}\frac{1}{s+(1-f)+fhD(f)}\frac{1}{N_g+N_h} \quad \text{and} \qquad \text{Eq. (S3a)}$$

$$\frac{1}{H_g}\frac{D(f)}{s+(1-f)+fhD(f)}\frac{1}{N_g+N_h}, \text{ respectively.} \qquad \text{Eq. (S3b)}$$

In general, the number of herbivores depends on the patch size, i.e. N_g and N_h; therefore, we represent the number of herbivores within the patch as $M(N_g, N_h)$. By multiplying Eq. (S3a) and (S3b) by $M(N_g, N_h)$ and the level of damage caused per attack, we obtain the total damage to glabrous and hairy plants, respectively. We defined ε as the quotient of damage per attack divided by the handling time of glabrous plants H_g; then, we can represent the damages

772 to glabrous and hairy plants using Eq. (4a) and (4b), respectively.

773

774 **Appendix S2. Factors responsible for the total amount of herbivory in a patch**

775 The number of herbivores usually shows a non-linear response to the number of plants in a
776 patch (e.g. a square-root response, Hambäck *et al.* 2014). If the mobility of herbivores is
777 limited during their life cycle, the current number of herbivores might also depend on their
778 past abundance or fecundity in a patch. In this section, we first investigated the relationship
779 between the number of plants and their leaf damage, which reflects the amount of resource
780 acquired for herbivores. We also examined the relative importance of the past abundance of
781 herbivores and the frequency of hairy plants on the total amount of leaf damage. Finally, we
782 ran the MCMC estimation assuming a square-root response of the number of herbivores to
783 patch sizes and compared the results with those estimated by assuming a linear response of
784 the number of herbivores to patch sizes. For this line of analyses, we collected the leaf
785 damage data as we monitored the number of hairy and glabrous plants in the field site during
786 the 4-year observations (described in the main text). We evaluated the proportion of the lost
787 leaf area for all individual plants in each patch using the same procedure as that described by
788 Sato and Kudoh (2016).

789 We first determined a relationship between the total amount of damage and the
790 number of plants in a patch as: Damage = $a \times N^b$, where N is the number of plants. The nls
791 function in R was used to estimate the parameters a and b. This non-linear regression showed
792 that the scaling parameter did not differ from 1 (b = 1.11 ± 0.17 [± 95% CI], t = 12.8, P <
793 10^{-16}). The coefficient a was 0.08 ± 0.02 [± 95% CI; t = 3.96, P < 0.001] in the non-linear
794 regression. This results support our basic model assuming a linear response of *P. brassicae* to
795 the number of *A. halleri* plants in a patch.

We then examined the factors responsible for the total amount of damage in a current year. A multiple regression was applied because of the linearity between the total damage and the number of plants. The response variable was the total amount of leaf damage in a current year in a patch, and the explanatory variables were the current number of plants, current frequency of glabrous plants, and the total amount of leaf damage in the previous year. As a result, the current amount of damage was positively correlated with the current number of plants and the previous amount of leaf damage (coefficient ± SE = 0.10 ± 0.01 and 0.15 ± 0.04, t = 11.8 and 3.6, $P < 10^{-16}$ and < 0.001, respectively), but not with the current frequency of glabrous plants (coefficient ± SE = 0.004 ± 0.21, t = 0.02, P = 0.98). This result indicates a negligible effect of the morph-frequency on the amount of herbivory. Despite the statistical significance of the past leaf damage, the current number of plants contributed more to the current amount of damage (R^2 = 0.29 and 0.04 for the current number and past damage, respectively). Temporal dynamics of herbivory was therefore unclear in the present field data on *A. halleri*.

To test how the assumption about the number of herbivores alters the fitting of our plant demography model to the field data, we additionally ran MCMC with the square-root number of plants (see "***Statistical analysis***" of **"Plant demography"** in the main text). This square-root response of herbivores has specifically been reported as a resource dilution effect on herbivory (Hambäck *et al.* 2014). The calculated log-likelihood was, however, smaller (-736.7 [-741.1 to -734.8], median and 95% CI) than the MCMC with the non-transformed number of plants in a patch, thus assuming the square-root relationship between the number of herbivores and the number of plants impaired the fitting to the field data.

Appendix S3. Likelihood of the observed dynamics of a plant patch

Hereafter, we refer to a patch where the number of glabrous and hairy plants is N_g and N_h, respectively, as $\{N_g, N_h\}$. First, we consider the probability $\mathrm{Pr_D}$ that the patch $\{N_g, N_h\}$ becomes $\{n_g, n_h\}$ after the death stage. If we assume that the death event of each plant occurs independently, the number of dead plants follows a binomial distribution whose probability is the mortality, as expressed in Eq. (6a) and (6b). Then, we can calculate the probability $\mathrm{Pr_D}$ by multiplying the two probability mass functions of the binominal distribution, i.e.

$$\mathrm{Pr_D}(\{ n_g, n_h \}|\{N_g, N_h\}) = \binom{N_g}{n_g} \mu_g^{N_g - n_g} (1-\mu_g)^{n_g} \binom{N_h}{n_h} \mu_h^{N_h - n_h} (1-\mu_h)^{n_h} , \quad \text{Eq. (S4a)}$$

where μ_g and μ_h are the mortality of glabrous and hairy plants, i.e. Eq. (6a) and (6b), respectively, and

$$\binom{n}{k} = \frac{n!}{k!(n-k)!} . \quad \text{Eq. (S4b)}$$

Next, we focus on the probability $\mathrm{Pr_R}$ that a patch $\{n_g, n_h\}$ becomes $\{N'_g, N'_h\}$ after the recruitment stage. Here, we assume that the probability distribution of the number of established individuals follows a Poisson distribution with the mean number of glabrous (λ_g) and hairy plants (λ_h) equal to Eq. (8a) and (8b), respectively. Therefore, the probability $\mathrm{Pr_b}$ is represented as

$$\mathrm{Pr_R}(\{N'_g, N'_g\}|\{ n_h, n_h \}) = \frac{\lambda_g^{N'_g - n_g} e^{\lambda_g}}{N'_g - n_g} \frac{\lambda_h^{N'_h - n_h} e^{\lambda_h}}{N'_h - n_h} \quad \text{Eq. (S5)}$$

Summing up the probabilities of all possible outcomes of death and recruitment stages, we obtain the probability that the patch $\{N_g, N_h\}$ becomes $\{N'_g, N'_h\}$ at the next time step, i.e.

$$\sum_{n_g=0}^{N_g}\sum_{n_h=0}^{N_h}\left[\Pr_D\left(\{N_g-n_g, N_h-n_h\}\,|\,\{N_g, N_h\}\right)\Pr_R\left(\{N'_g, N'_j\}\,|\,\{N_g-n_g, N_h-n_h\}\right)\right] \quad \text{Eq. (S6)}$$

By using a certain parameter set, Eq. (S6) provides the probability that a given plant patch $\{N_g, N_h\}$ turns into another state $\{N'_g, N'_h\}$ during a year. Multiplying the probabilities over a set of patches, we obtain the likelihood, i.e. a joint probability, to show how likely the observed dataset for patch dynamics in the field is in the plant demography model consisting of death (binomial error) and recruitment (Poisson error) events.

Appendix S4. The influence of patch size on invasion difficulty

Our basic model assumed that all seeds or clones reached to a given patch can be established and mature at the patch. However, because of the spatial or resource limitation of the patch, the patch size might reduce the probability that seeds or clones successfully establish themselves and mature in the focal patch. To investigate such a density effect, let us consider a situation in which the probability of being established decreases linearly with the number of mature plants at the focal patch, i.e. the established probability is $1 - k\,(n_g + n_h)$, where k is the coefficient of the mature plant number effect. Then, the mean number of glabrous and hairy plants established at a patch $\{n_g, n_h\}$ is

$$\lambda_g = \left(1-k(n_h+n_g)\right)\left(d\tilde{R}_g + (1-d)R\left[1-\frac{v}{s+(1-f)+fhD(f)}\right]n_g\right) \quad \text{and} \quad \text{Eq. (S7a)}$$

$$\lambda_h = \left(1-k(n_h+n_g)\right)\left(d\tilde{R}_h + (1-d)R\left[1-\frac{vD(f)}{s+(1-f)+fhD(f)}-c\right]n_h\right), \quad \text{Eq. (S7b)}$$

respectively.

We applied MCMCs to the extended model in addition to the six demographic

parameters (v, R, c, d, m_c, and m_d). The parameter k was estimated as an exponential scale to examine the range of positive values. As described in the main text, we ran eight independent chains of 10^6 iterations of MCMCs with a 10^5 burn-in period and 10^3 thinning interval. The initial parameters of k and m_d were set to 0.01, and the same initial constraints, as shown in the main text, were applied to the remaining five parameters. According to the MCMC analysis, the effect of the mature plant k was estimated at nearly zero ($k < 0.01$ at upper 95% CI). The estimated values of the other six parameters (v, R, c, d, m_c, and m_d) and the log-likelihood were the same as those of the model without k up to the level of second significant figures. Thus, plant density had negligible effects on the recruitment process.

Appendix S5. Importance of ODC in model predictions

In this section, we investigated the effects of the defensive advantage of hairy plants, i.e. the relative energy intake e and the relative handling time h, on parameter estimations and model predictions. First, we focused on the parameter estimation of ODC (e, h, s, and α) from the leaf damage data obtained in the laboratory experiment. Although we estimated these parameters assuming that both energy intake and handling time were different between the herbivory of glabrous and hairy plants, either of them might be the same between the two plant morphs (i.e. the situation that $e = 1$ or $h = 1$). However, the estimated damage to hairy and glabrous plants were insufficiently fitted to the average damage estimated from the empirical data when $e = 1$ or $h = 1$ (Figure S1). These results indicate that incorporating both the increment of handling time and the decrement of energy intake is necessary to explain the observed pattern of leaf damage.

Next, to examine the effects of ODC on model predictions, we investigated the simulation model assuming no difference between glabrous and hairy plants in both energy

intake and handling time (i.e. $e = h = 1$). In such a situation, the probability of feeding on hairy plants D is always one, and the damage to glabrous and hairy plants, Eq. (5a) and (5b), becomes a constant value $v/(s+1)$ regardless of the frequency of hairy plants f. Then, the mortality of glabrous and hairy plants, Eq. (6a) and (6b), can be represented by a constant value m. Similarly, the total number of offspring of hairy and glabrous plants, Eq. (8a) and (8b), can be rewritten as

$$d\tilde{R}_g + (1-d)R'n_g \quad \text{and} \qquad \text{Eq. (S8a)}$$

$$d\tilde{R}_h + (1-d)(R'-c')n_h, \qquad \text{Eq. (S8b)}$$

respectively, where $R' = R(1-v/(s+1))$ and $c' = Rc$.

We ran eight independent chains of 10^6 iterations of MCMCs with a 10^5 burn-in period and 10^3 thinning interval to estimate the parameters R', c', d, and m (Table S1). We used deviance information criteria (DICs; Spiegelhalter *et al*. 2002 *J. R. Stat. Soc. Series B*, **64**, 583–639) to compare predictability of the models with and without ODC. The DICs were calculated on the basis of the mean and variance of log-likelihoods from the MCMCs. The model with ODC showed a smaller DIC (= 1474.2) than the model without ODC (= 1484.1), indicating the importance of ODC in predicting the observed demography. The result that the defence cost c was near to and overlapped with zero (Table S1a) showed that this cost needs not be considered in the absence of ODC. Additionally, we estimated the model including the carrying capacity based on a similar extension in Appendix S4. However, the inverse of carrying capacity k exhibited nearly zero values and the results of the remaining parameters were almost the same to those in the case without carrying capacity (Table S1b).

Table S1 MCMC estimates for the model parameterised without ODC by herbivores ($h = 1$ and $e = 1$). The results of the estimates without **(a)** and with **(b)** the inverse of the carrying

910 capacity *k* are shown. The median with 95% lower and upper percentiles is shown for
911 parameter distributions.

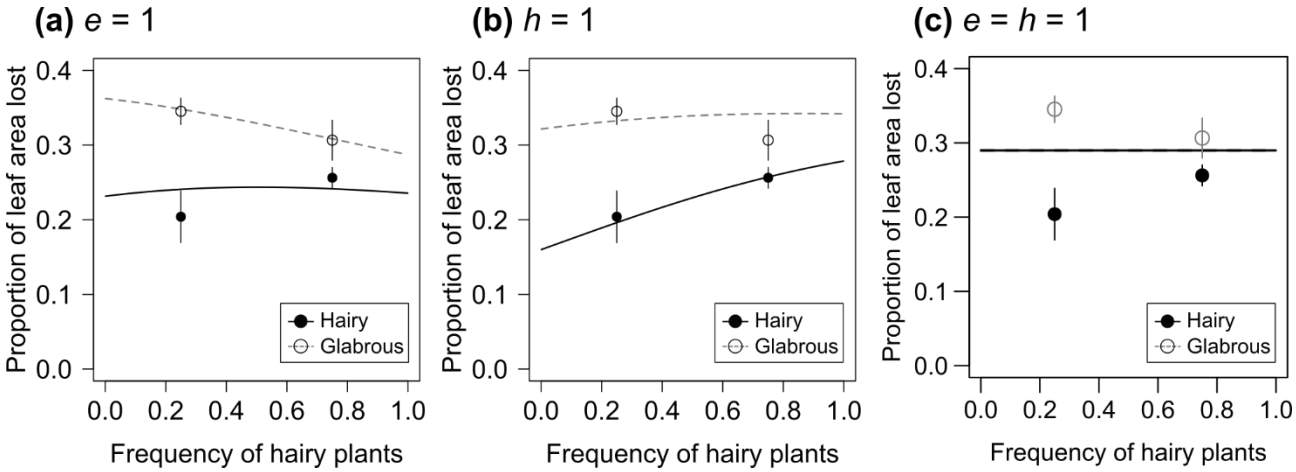
(a) Without carrying capacity

Parameter	Description	Percentiles		
		Median	2.5%	97.5%
R'	Transformed growth rate	1.79	1.38	2.34
c'	Transformed cost of defence	-0.03	-0.41	0.35
d	Proportion of interpatch dispersal	0.11	0.08	0.14
m	Mortality	0.75	0.69	0.80
---	*Log-Likelihood*	*-738.0*	*-741.9*	*-736.5*

(b) With carrying capacity

Parameter	Description	Percentiles		
		Median	2.5%	97.5%
R'	Transformed growth rate	1.79	1.38	2.32
c'	Transformed cost of defence	-0.03	-0.40	0.34
k	Inverse of carrying capacity ($0<k<1$)	$<10^{-21}$	$<10^{-67}$	$<10^{-3}$
d	Proportion of interpatch dispersal	0.11	0.08	0.14
m	Mortality	0.75	0.69	0.80
---	*Log-Likelihood*	*-738.0*	*-741.7*	*-736.6*

912
913
914
915



916 **Figure S1** Prediction of leaf damage (evaluated by the proportion of leaf area lost to
917 herbivory; mean ± SE) in hairy (solid line with closed circles) and glabrous (dashed line with
918 open circles) plants in a laboratory by using ODC model. Panels (a), (b), and (c) present the
919 results when no differences were assumed between hairy and glabrous plants in energy intake
920 (i.e. $e = 1$), handling time ($h = 1$), and both ($e = h = 1$), respectively. However, the estimated
921 leaf damage of hairy and glabrous plants (solid and dashed lines) cannot explain the leaf
922 damage observed in empirical data (open and closed circle, respectively). Parameter values
923 are at $h = 2.10$, $s = 1.76$, $\alpha = 0.95$ in the case $e = 1$; $e = 0.32$, $s = 2.11$, $\alpha = 1.49$ in the case $h =$
924 1; and $s = 2.456$, $\alpha = 0.685$ in the case $e = h = 1$.
925
926
927
928
929
930
931
932
933

Appendix S6. The condition for the coexistence of hairy and glabrous plants

First, we calculate the frequency of hairy plants at a stable state. The relative amounts of offspring per one glabrous and one hairy plant are obtained by dividing Eq. (7a) and (7b) by Rn_g and Rn_h, respectively, i.e.

$$1-\frac{v}{s+(1-f)+fhD(f)} \quad \text{and} \qquad \text{Eq. (S9a)}$$

$$1-\frac{D(f)}{s+(1-f)+fhD(f)}-c. \qquad \text{Eq. (S9b)}$$

Because herbivory has negligible effects on mortality (m_d; see Table 1 in the main text), glabrous and hairy plants differ only in the damage and the cost of defence in this model. The fitness of glabrous and hairy plants are, therefore, proportional to Eq. (S9a) and (S9b), respectively. By solving the condition that Eq. (S9a) is equal to Eq. (S9b), we can investigate the frequency of hairy plants at a stable state, i.e.

$$\frac{v}{s+(1-f^*)+f^*hD(f^*)}=\frac{vD(f^*)}{s+(1-f^*)+fhD(f^*)}+c. \qquad \text{Eq. (S10)}$$

By solving Eq. (S10), we obtained the equilibrium frequency of hairy plants f^* at the stable state.

Next, we investigated the condition for the coexistence of hairy and glabrous plants. Let us consider the situation that population is filled by glabrous plants, i.e. $f = 0$. For the invasion of the population by hairy plants, the number of offspring of hairy plants per capita should be larger than that of glabrous plants, i.e.

$$1-\frac{v}{s+1}>1-\frac{vD(0)}{s+1}-c. \qquad \text{Eq. (S11)}$$

By solving this, we obtain

$$1<\frac{1}{1+\exp[\alpha f^*]}+\frac{c(s+1)}{v} \quad . \qquad \text{Eq. (S12)}$$

If Eq. (S12) is satisfied, a hairy plant can potentially invade the population of glabrous plants. Similarly, for the invasion of the population of hairy plants by glabrous plants, the number of offspring of glabrous plants per capita should be larger than that of hairy plants, i.e.

$$1-\frac{v}{s+hD(1)}>1-\frac{vD(1)}{s+hD(1)}-c. \qquad \text{Eq. (S13)}$$

By solving this, we obtain

$$1<\frac{1}{1+\exp[-\alpha(1-f^{*})]}\left(1+\frac{c}{v}\right)+\frac{cs}{v}. \qquad \text{Eq. (S14)}$$

If Eq. (S14) is satisfied, a glabrous plant can potentially invade the population of hairy plants. Consequently, two morphs can coexist if both Eq. (S12) and Eq. (S14) are simultaneously satisfied.